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Note

Esterification and etherification by silver oxide–organic halide reaction gas chromatography

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Fatty acids are usually esterified prior to their separation by gas chromatography (GC) because of difficulties associated with adsorption of the acids on the chromatographic support[1]. Also, GC separation of esters can be performed on liquid phases of a wider range than can be used for the separation of the free acids. Long-chain fatty acids are usually converted to their methyl esters[1] while the corresponding short-chain acids may be esterified with propanol[2] or other higher alcohols[3–7].

As part of a programme on mutton flavour research[8], we had the problem of obtaining the chemical identity of the unusual medium-chain (C_6–C_{12}) volatile fatty acids of mutton fat after GC separation and sensory evaluation. A method was required to isolate the individual compounds, convert them into methyl esters and re-chromatograph the esters for characterisation by GC–mass spectrometry (MS). Because of the submicrogram quantities involved, an "on-column" technique in which the operations of trapping, esterification and introduction for re-chromatography could be combined in the same apparatus was mandatory.

The reactions of methyl iodide with silver salts of acids and of methyl iodide–silver oxide with alcohols are used as standard methods for the preparation of methyl esters and methyl ethers, respectively[9,10]. Based on these reactions, the present paper describes a technique in which a silver oxide-packed tube was used for trapping fatty acids after their separation by GC and methylation was carried out *in situ* by subsequent introduction of methyl iodide. The tube was then used for introduction of the sample into a second gas chromatograph or into a GC–MS system. The same tube could also be used for "on-column" methylation of the original mixture of acids, with subsequent analysis of the ester derivatives by GC. The effect of the silver oxide–methyl iodide combination on certain hydroxy compounds was also studied.

EXPERIMENTAL

Methyl iodide was distilled in a fume cupboard before use (the reagent is carcinogenic[11]). Silver oxide was deposited by precipitation from silver nitrate and an excess of sodium hydroxide onto 40–60 mesh Celite to give 10% (w/w) of the oxide. The coated material was washed with distilled water until the washings were neutral, and dried overnight at 100°.

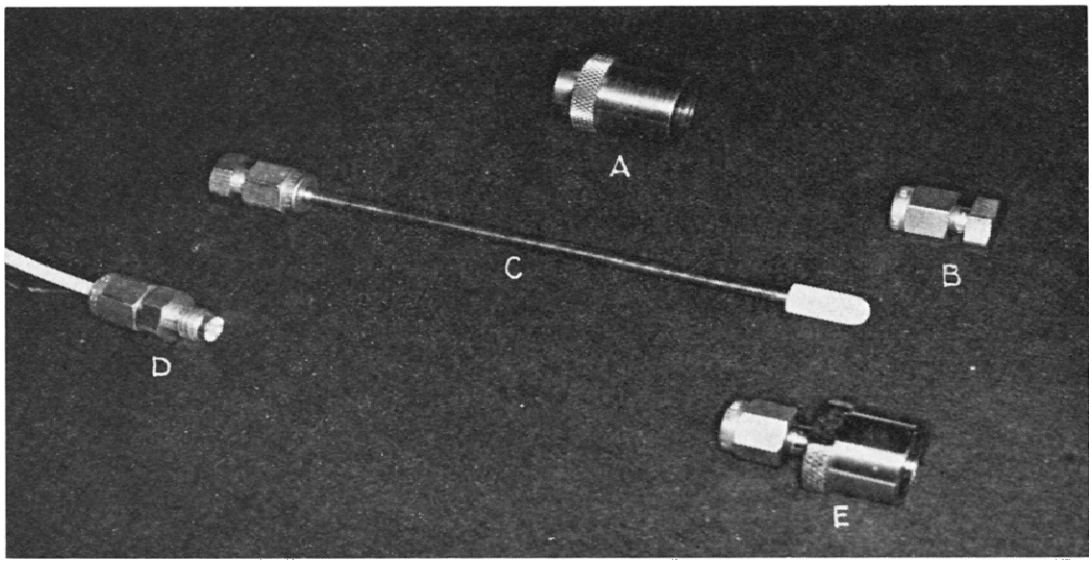

Fig. 1. Modified septum holder and associated equipment for transfer of samples from traps onto a GC column for the Hewlett-Packard chromatograph. A = Original septum holder; B = Swagelok cap and nut with PTFE ferrules ($\frac{1}{8}$ in.); C = trap with Swagelok cap ($\frac{1}{8}$ in., metal ferrules) and partially drilled PTFE rod (for storage of samples) D = external gas supply; E = modified septum holder with Swagelok nut ($\frac{1}{8}$ in.).

The traps were constructed of 150 × 3.2 mm O.D. stainless-steel tubes packed 25 mm of their length at the collection end with the silver oxide reagent. The other end of the tube was fitted with an $\frac{1}{8}$-in. Swagelok cap and ferrules (Fig. 1C). Before use, the packed traps were heated to 200° for 1 h with a slow passage of nitrogen.

Steam volatile fatty acids were isolated from mutton fat as described previously[8]. The acids were separated on a 2.5 m × 3.2 mm O.D. stainless-steel column containing 10% (w/w) stabilised polyethylene glycol adipate (EGA; Analabs, North Haven, Conn., U.S.A.) on 100–120 mesh Gas-Chrom Q (Applied Science Labs., State College, Pa., U.S.A.) in a Hewlett-Packard Model 7620A gas chromatograph. The exit of the column was attached to a 5:1 splitter connected to a heated outlet (five parts) maintained at 200°. The acids were collected at room temperature from the gas chromatograph in the silver oxide-packed tubes so that the acids condensed on the oxide.

Esterification of trapped acids was accomplished by injecting 1 μl methyl iodide into the silver oxide reagent followed by heating the reagent-filled portion on a hot-plate at 100° for 2 min after the trap had been sealed at both ends by Swagelok caps. For the "on-column" methylation of a mixture of acids in solution, an aliquot of the solution was injected onto the reagent, followed by the methyl iodide. A new, freshly prepared trap was required for each sample because no reaction took place when a trap was used for a second collection–esterification.

The esterified contents of the traps, after cooling to dry-ice temperature, were injected onto a GC column in the Hewlett-Packard instrument through a modified septum retainer having a Swagelok thread attached (Fig. 1E). The holder was drilled to allow the trap to pass through it into a wide-bore column insert liner (that is normally used with the pyrolysis or solid sample injection units) in the injection port (at

200°) of the gas chromatograph. The trap was held in place by an $\frac{1}{8}$-in. Swagelok nut with PTFE ferrules on the holder. An external gas supply (*ca.* 20 lbs./sq.in., to yield a flow-rate of 45 ml/min through the column at room temperature) was connected to the trap to flush its contents onto the column, which was cooled 5–10 cm of its length at the injection port with dry ice. During connection of the trap to the gas chromatograph, the external gas supply was regulated to give a flow-rate of 10 ml/min through the cooled trap. Absence of this gas flow resulted in loss of sample, probably by blow-back into the cold part of the external gas line. Normal flow-rate of carrier gas through the column during the transfer of sample was reduced to 10 ml/min. The transfer of sample was allowed to proceed for 2.5 min, after which the normal carrier gas flow was established, the external gas supply was shut off and the oven of the gas chromatograph was rapidly heated to 50° for initiation of the temperature programme. Chromatography of the esters was carried out on the EGA column, or on a similar column containing silicone OV-101, with a standard temperature programme of 50°–220° at 2°/min. Similar equipment and procedures were used for transfer of samples to a Pye 104 gas chromatograph coupled by a silicone elastomer membrane interface to an AEI MS-30 mass spectrometer. Helium (40 ml/min) was used as the carrier gas for the mass spectral runs and nitrogen (50 ml/min) for all others.

RESULTS

Typical gas chromatograms of a mixture of standard *n*-fatty acids and of the methyl esters formed from them by the silver oxide–methyl iodide method are shown in Fig. 2.

The reaction appeared to go to completion under the described conditions,

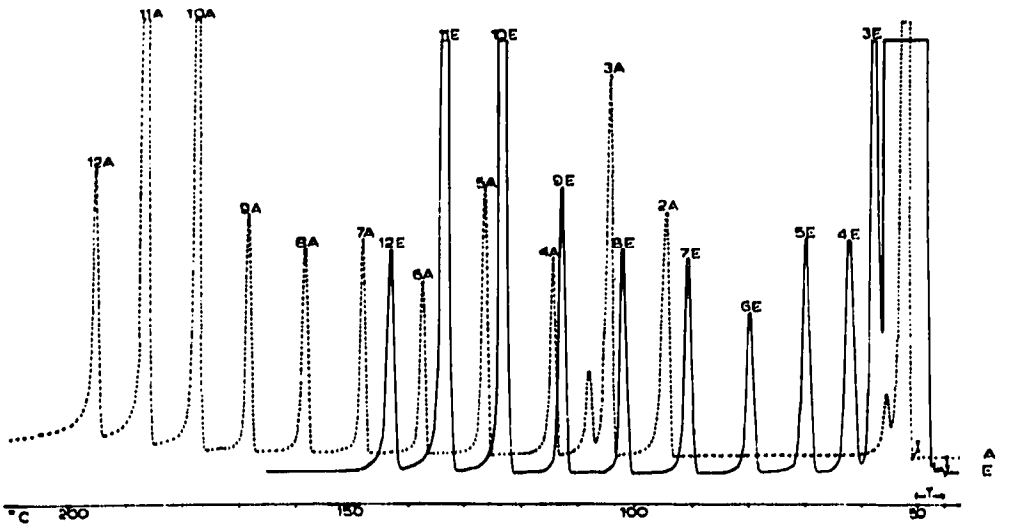

Fig. 2. Composite gas chromatograms on the EGA column of standard normal fatty acids (A) and their methyl esters (E) formed by the silver oxide–methyl iodide esterification of the acids. Peak numbers refer to the chain length of the acids. T = Transfer period; I = injection point.

acids were not apparent in the ester chromatograms and the relative peak sizes within the chromatograms were similar. Also, no degradation was apparent since chromatograms of esterified single acids did not contain peaks of lower homologues. The first peak to appear on the "solvent" tail on either the polar or the non-polar column was methyl propionate or methyl butyrate. Methyl formate (if it was formed) and methyl acetate were "lost" under the "solvent" peak. This "solvent" peak could be reduced by passing carrier gas through the trap at 10 ml/min at dry-ice temperatures for up to 1 min, but various amounts of the low-molecular-weight esters up to methyl caproate were lost at the same time.

An example of the use of the technique for the collection–esterification–rechromatography of fatty acids from the mutton flavour study is shown in Figs. 3 and 4. Acids of sensory interest, corresponding to small areas of the chromatogram on EGA (numbered in Fig. 3), were collected on the silver oxide reagent, esterified and introduced into the GC–MS system. Tracings of the esters from the GC detector and the total ion monitor of the mass spectrometer closely followed each other (Fig. 4).

The effect of the silver oxide–methyl iodide reagent on a limited number of hydroxy compounds was studied. Octanol was converted by the reagent to methyl octyl ether and 2-octanol was similarly converted to 2-methoxyoctane without formation of the corresponding fatty acid methyl ester, aldehyde or ketone. Identity of the methyl esters was confirmed by their mass spectral patterns. Both β-hydroxybutyric acid and methyl β-hydroxybutyrates were converted to methyl β-methoxybutyrate by the reagent, with a trace of methyl crotonate produced by dehydration of the hydroxy compounds (Table I). Butyrolactone was converted to methyl γ-methoxybutyrate by the reagent.

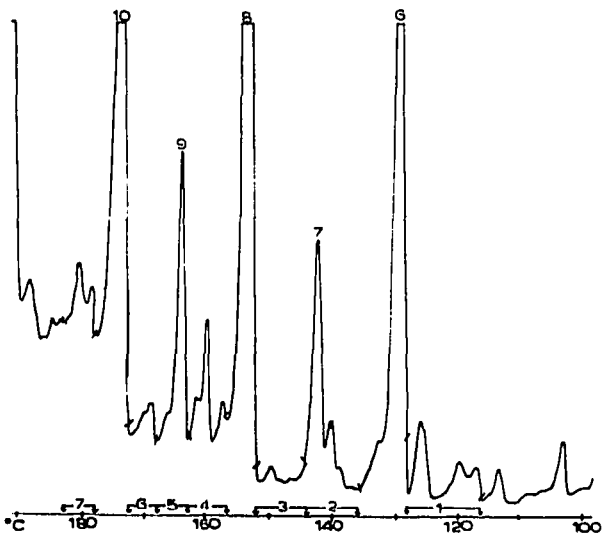

Fig. 3. Partial gas chromatogram on the EGA column of steam volatile fatty acids from a mutton mince cook-up showing areas collected in silver oxide traps. Peak numbers refer to the chain-lengths of the normal fatty acids.

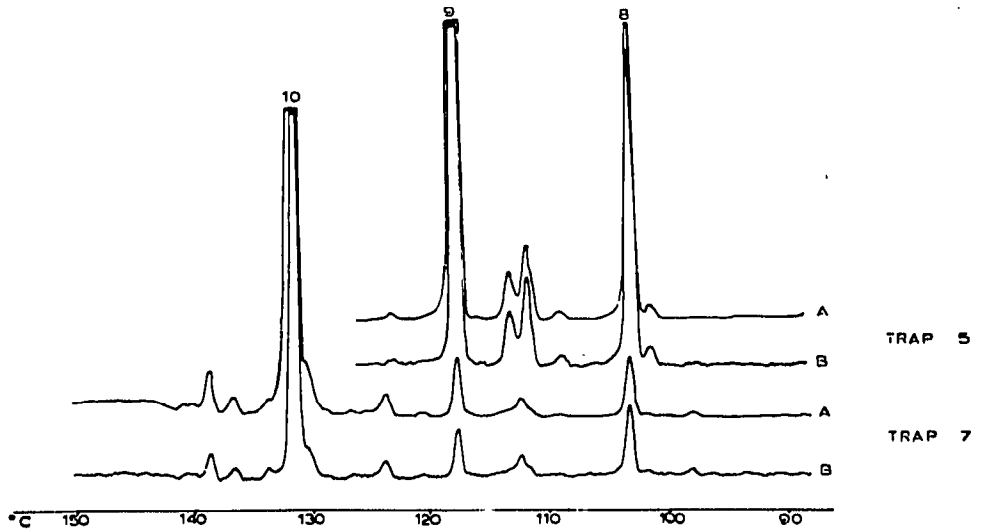

Fig. 4. Partial gas chromatograms on the OV-101 column of methyl esters from acids in traps 5 and 7 (Fig. 3). (A) Trace from total ion monitor of the mass spectrometer. (B) Trace from the flame ionisation detector of the gas chromatograph. Peak numbers refer to the chain-lengths of the normal fatty acids.

TABLE I

GC AND MS DATA FOR HYDROXYBUTYRATE DERIVATIVES AND THEIR Ag_2O/CH_3I REACTION PRODUCTS

Compound	*ECL (on EGA)*[16]	*Major MS peaks (relative intensities in parentheses)*
Butyrolactone	10.6	86 (53), 67 (38), 55 (67), 53 (100)
Methyl γ-methoxybutyrate	7.5	117 (4), 101 (54), 87 (5), 85 (7), 74 (100)
Methyl β-hydroxybutyrate	8.7	117 (0.5), 103 (8), 100 (4), 87 (9), 85 (5), 75 (2), 74 (32)*, 45 (44), 43 (100)
Methyl β-methoxybutyrate	6.8	131 (0.2), 117 (6), 102 (7), 101 (6), 87 (3), 85 (6), 75 (23)*, 74 (0.9), 59 (100)
Methyl crotonate	5.2	100 (90), 85 (100), 59 (32)

* Ref. 17.

DISCUSSION

GC separation and structural analysis of fatty acids by mass spectrometry is facilitated by conversion of the acids into the corresponding esters[1,12]. Acids are poorly transmitted (compared with the esters) through the elastomer interface of the GC–MS system. Extensive MS data have been recorded on methyl esters of fatty acids[12]. Reaction of methyl iodide with silver salts of fatty acids has been recommended for samples containing a wide range of acids, *e.g.* milk fats[13]. This method, suitably modified, proved useful here for the derivatisation of acids collected from the GC column and for other small quantities of acids or alcohols. The reagent, when used under the conditions described, did not appear to affect the basic structure of normal,

branched-chain and unsaturated fatty acids, but opening of a γ-lactone ring to yield a methoxy ester was shown to take place. Dehydration of hydroxy compounds, where the result was a conjugated system of double bonds, also took place to a limited extent.

The apparatus and procedures described in this work can be recommended as a general and useful technique for the "on-line" esterification and etherification of sub-microgram quantities of fatty acids and hydroxy compounds as a prelude to their GC analysis. The use of the system for both trapping and sample introduction, as illustrated by the mutton flavour work, should find other applications in situations where trace components in complex mixtures of these compounds need to be concentrated and further separated prior to their characterisation by GC or GC–MS means. Similar types of apparatus to that described here have been developed for small-scale hydrogenation[14,15] and ozonolyses[15].

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